White Eye Color Mutant in *Haematobia irritans* (Diptera: Muscidae)

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ABSTRACT The wild-type eye color of the horn fly, *Hematobia iritans* (L.) (Diptera: Muscidae), is a dark reddish brown. An apparent spontaneous mutation in a single adult colony fly resulted in a white-eyed mutant. A colony of white-eyed horn flies was established from this single individual and has been maintained in the laboratory. Laboratory crosses determined that the white-eyed phenotype is inherited as a simple Mendelian autosomal recessive with complete penetrance. No other differences from the wild-type flies were detected in the external characteristics of the mutant phenotype or in egg viability. However, white-eyed flies had significantly lower amounts of the pigment dihydroxyxanthommatin in their heads, suggesting either the lack of xanthommatin production, or a failure of transport and storage within the head of the mutant phenotype.

KEY WORDS *Hematobia irritans*, mutant, eye color

The horn fly, *Hematobia irritans* (L.), is one of the most damaging obligate ectoparasites of cattle throughout North and South America. Because of the adverse economic impact on the cattle industry, horn flies have been reared in vitro since 1961 for research purposes at the Knipling–Bushland U.S. Livestock Insects Research Laboratory, Kerrville, TX (Harris 1962). The occurrence of eye color mutants in dipterans is well documented (Wagoner 1969, Sharp and Chambers 1973, Rössler and Koltin 1976, D'Haeseleer et al. 1987, Taylor and Cuevas 1986, Rössler and Rosenthal 1988, McCombs and Saul 1989, Challoner et al. 1997, Rasgon and Scott 2004); however, to date, no eye color mutants have been described for *H. irritans.* The characterization of a visible genetic marker such as an eye color mutation in an economically important species such as the horn fly could prove valuable for behavior and population dynamic studies as well as release and recapture studies. In this article, we report the isolation, inheritance pattern, egg viability, and potential biochemical basis for a white eye mutant in a laboratory population of horn flies.

Materials and Methods

Establishment of White-Eyed Colony. A white-eyed mutant male adult occurred as a spontaneous mutation in a laboratory culture of horn flies in September 1997. The wild-type eye color of adult horn flies is a dark reddish brown (Fig. 1). No other visible differences were detected in the external characteristics of the mutant phenotype.

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Laboratory colonies of the horn fly were maintained according to the methods described by Kunz and Schmidt (1985). A colony of white-eyed horn flies was established by allowing the single adult male collected in September 1997 to mate with females from the laboratory colony. The male was mated with 30 wild-type females that were ≈4 d posteclosion and had possibly been previously mated, because no virgin females were available at the time the white-eyed male was collected. This mating yielded 114 adult flies, all of which had wild-type eye color. These flies were then allowed to interbreed and produced 523 offspring of which 109 (60 males and 49 females) had white eyes. These white-eyed adults were used to establish a colony of horn flies with white eyes, and the mutant phenotype has persisted in the colony for >8 yr.

Determination of Inheritance Pattern of White-Eyed Mutation. Four different types of crosses were performed to determine the genetic nature of the white-eye mutation (Table 1). For each cross, 50 males and 50 females were mated. Cups of horn fly larval media (Lohmeyer and Kammlah 2006) were then seeded with 100 randomly selected eggs from each cross. Pupae were recovered, and adult flies were scored for eye color and sex. To determine whether the white eye type is an X-linked trait, wild-type eye color males were crossed with white-eye color females.

To assess whether the white eye color type is inherited as an autosomal trait, 50 homozygous wild-type males were crossed with 50 white-eyed females to generate heterozygous offspring. From these offspring, 50 males and 50 females were then intercrossed. For each of the six crosses (Table 2), horn fly

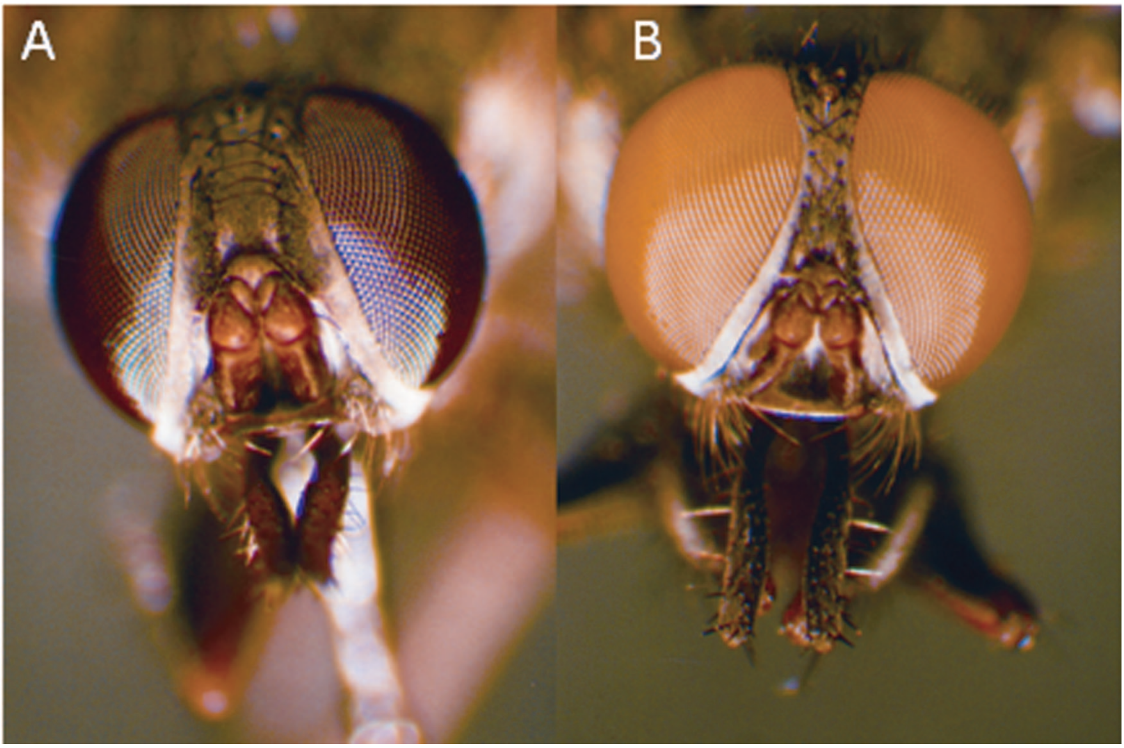

Fig. 1. Eye color of wild-type *H. irritans* (A) and the white-eyed color mutant (B).

larval media were seeded with 100 randomly chosen eggs, and the resulting progeny were scored for eye color and sex.

Egg Viability. Egg viability was determined for each of the four crosses performed in the first test (Table 1). To determine viability, three sets of 100 eggs were randomly selected from each cross. Eggs were held on moist filter paper and observed for hatching. Percentage of egg hatch was calculated by dividing the number of eggs that hatched by the total number of eggs.

Pigment Analysis. Xanthommatin, an ommochrome pigment, is used for light perception and is common among many insect species (Robinson 1971). Differences in levels of this pigment have been reported as the biochemical basis for eye color mutations for several species of insects (D'Haeseleer et al. 1987, Challoner and Gooding 1997, Wraight et al. 1999, Rasgon and Scott 2004). To determine the basis for the eye color variation in the white-eyed horn flies, a modified version of the procedure for quantifying dihydroxyxanthommatin as described by Linzen and Schartau (1974) was followed. The amount of dihydroxyxanthommatin present in each eye phenotype was ascertained as follows. Two heads of each phenotype

Table 1. Segregation of eye color phenotypes in F_1 progeny of *H. irritans*

Cross	Parental eye phenotype (Male × Female)	Offspring phenotype	No. observed[a]	No. expected[b]	χ^2	P
1	Wild × wild	White	0	0	0	>0.9995
		Wild	180	180		
2	Wild × white	White	0	0	0	>0.9995
		Wild	184	184		
3	White × wild	White	0	0	0	>0.9995
		Wild	125	125		
4	White × white	White	145	145	0	>0.9995
		Wild	0	0		

[a] Data in table are a total of three separate matings of 50 females and 50 males for each type of cross; 100 eggs were randomly selected from each mating.
[b] The expected numbers are based on the assumption that the parents were homozygous.

Table 2. Determination of the autosomal recessive nature of the white-eye phenotype in *H. irritans*

Cross[a]	Phenotype	No. observed[b]	No. expected[c]	χ^2	P
1	White	9	10	0.13	$0.8 > P > 0.7$
	Wild	31	30		
2	White	10	12	0.44	$0.6 > P > 0.5$
	Wild	38	36		
3	White	17	16.75	0.01	$0.95 > P > 0.9$
	Wild	50	50.25		
4	White	17	15	0.36	$0.6 > P > 0.5$
	Wild	43	45		
5	White	6	6.25	0.01	$0.95 > P > 0.9$
	Wild	19	18.75		
6	White	8	11.75	1.60	$0.2 > P > 0.3$
	Wild	39	35.25		

[a] Crosses 1–6 represent intercrosses of heterozygous adults.
[b] Data in table are from matings of 50 females and 50 males; 100 eggs randomly selected after each mating.
[c] Expected values were generated by assuming a Mendelian 3:1 ratio.

were homogenized individually in 400 μl of 0.5% ascorbic acid in 1 M HCl. To each homogenate, 400 μl of *n*-butanol was added. The homogenates were then centrifuged, and the butanol fractions were removed and run in a spectrophotometer (EL 808 BioTek Instruments, Winooski, VT) at 490 nm. This procedure was repeated 20 times for each phenotype.

Results and Discussion

Crossing the white-eyed mutant horn flies with white-eyed mutant horn flies always produced white-eyed offspring (Table 1, cross 4). The results of cross two indicate that the white-eye mutation is not an X-linked trait. If the white-eye mutation was sex-linked, then all resulting female progeny would have wild-type eyes and all resulting male progeny would be white-eyed. Crosses were then conducted to test for the possibility of autosomal inheritance.

A Mendelian ratio of three wild-type to one white eye was observed for all six of the crosses (Table 2). The results of these crosses show that the white eye phenotype is inherited as a simple Mendelian autosomal recessive trait affecting eye color in adult horn flies.

No difference in egg viability was observed for any of the four crosses tested ($F = 0.50$; df = 6, 3; $P > 0.05$). This suggests that the white-eye mutation does not have an impact on viability of horn fly eggs.

The levels of dihydroxyxanthommatin were dramatically reduced in the heads of the white-eyed horn fly phenotype. Wild-type flies (13.074 μg per head, $S^2 = 5.344$) had significantly more dihydroxyxanthommatin present in their heads than did the white-eyed phenotype (1.2267 μg per head, $S^2 = 0.462$; $P = 4.11 \times 10^{-15}$). No differences in dihydroxyxanthommatin levels were detected between male and female flies of the same eye phenotype. Most ommochrome pigments are produced from the precursor tryptophan (Challoner and Gooding 1997). The white-eye mutation seen in adult horn flies may affect xanthommatin production (Challoner and Gooding 1997) or the transport and storage of the pigment within the head (Summers et al. 1982).

Although eye color mutations have been described for several orders of insects, including Diptera, to date no other eye color mutant has been described for the horn fly. The white-eye mutation in adult horn flies is easy to score visually. This type of visible mutation in an important economic pest may prove useful for population dynamics, behavior, and release and recapture studies. In addition, eye color mutations have previously been used as selectable markers for transformation in many insect systems (Sarkar and Collins 2000). Characterization of the white-eye mutation at the molecular level as well as additional fitness may lead to the use of the white-eyed mutation as a marker for transformation studies in horn flies.

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